

Applicants have listed herein each document of which they are aware that may be material to the examination of this patent application, and have provided a concise explanation of the relevance of each document listed.^a With the exception of the priority document of Japanese Patent Publications 67388/1977 and 146295/1987, (United States application Serial No. 572,008) which is enclosed herewith, a copy of each document has previously been provided to the Examiner in connection with the prosecution of the parent application, United States application Serial Number 496,915, now United States patent 4,711,955, issued December 8, 1987. For the convenience of the Examiner, a completed Form PTO-1449 listing each of these documents is attached.

United States Patents

^a 3,328,389	^a 4,228,237
3,337,530	^a 4,230,698
3,338,882	^a 4,230,797
3,804,826	^b 4,247,544
3,893,998	^b 4,261,893
3,915,958	4,267,171
3,917,583	4,302,204
3,960,840	^a 4,318,980
3,968,101	4,318,981
4,008,363	4,318,982
4,038,480	4,355,165
4,048,307	^a 4,358,535
^b 4,086,417	4,363,759

^a This document may be relatively more pertinent than other cited documents.

^b This document was cited by the United States patent examiner in the parent application hereto.

4,088,639	4,374,925
4,096,324	^a 4,380,580
4,134,792	^a 4,383,031
4,151,349	4,446,231
4,171,432	4,469,863
4,213,893	

United States Patent Applications

^b572,008

European Patent Applications

70685
70687

German Offenlegungsschriften

^{c,d} DE-A-2618419 (no translation available) [related to United States patents 4,230,797 and 4,261,893 and Great Britain patents 1,548,741 and 1,552,607]

^{d,e} DE-A-2618511 (no translation available) [related to United States patents 4,230,797, 4,261,893 and Great Britain patents 1,548,741 and 1,552,607]

DE-3045375-A1 (no translation available)

^a This document may be relatively more pertinent than other cited documents.

^b Japanese Patent Applications 67388/1977 and 146285/1987 (neither available) were cited by the Japanese Patent Office in an Office Action in a Japanese Patent application claiming priority from the parent application hereto. Both cited documents claim priority from the United States patent application cited herein.

^c This document was cited by the Canadian patent examiner in an application claiming priority from the parent application hereto.

^d This document was cited in the European Patent Office Search Report in an application claiming priority from the parent application hereto.

^e This document was cited by the European patent examiner in an application claiming priority from the parent application hereto.

United Kingdom Patent Application

^a2,019,408 A

2,026,690 A

2,034,323 A

2,041,922 A

2,045,239 A

2,125,964 A

Great Britain Patents

1,548,741

^b1,552,607

Japanese Patent Applications

43-24191 (no translation available)

58-62194 (no translation available)

PCT Patent Applications

^cWO83/02276 (no translation available)

^cWO83/02277 (no translation available)

WO83/02286

Other Documents

J. G. J. Bauman et al., "A New Method for Fluorescence Microscopical Localization of Specific DNA Sequences by In Situ Hybridization of Fluorochrome-Labelled RNA", Exp. Cell Res., 128, pp. 485-90 (1980) ["Bauman I"]

J. G. J. Bauman et al., "Rapid and High Resolution Detection of in situ Hybridisation to Polytene Chromosomes Using

^a This document may be relatively more pertinent than other cited documents.

^b This document, and an Israeli equivalent (Israel Patent No. 49354), was cited by the Israeli patent examiner in an application claiming priority from the parent application hereto.

^c This document was cited by the European patent examiner in an application claiming priority from the parent application hereto.

Fluorochrome-Labeled RNA," Chromosoma (Berl.) 84, pp. 1-18 (1981) ["Bauman I"]

^aE. A. Bayer and M. Wilchek, "The Use of the Avidin-Biotin Complex as a Tool in Molecular Biology," in Methods of Biochemical Analysis, 26, pp. 1-45 (1980)

^{ab}D. Bergstrom and M. Ogawa, "C-5 Substituted Pyrimidine Nucleosides. 2. Synthesis Via Olefin Coupling To Organo-palladium Intermediates Derived From Uridine and 2'-Deoxyuridine, J. Am. Chem. Soc., 100, pp. 8106-12 (1978) ["Bergstrom I"]

^aD. Bergstrom and J. L. Ruth, "Properties of C-5 Mercurred Pyrimidine Nucleosides," J. Carbohydrates, Nucleotides and Nucleosides, 4, pp. 257-69 (1977) ["Bergstrom II"]

^aC. F. Bigge et al., "Palladium-Catalyzed Coupling Reactions of Uracil Nucleosides and Nucleotides," J. Am. Chem. Soc., 102, pp. 2033-38 (1980)

^aC. Brandon et al., "Structure of a New Pyrimidine from Bacillus Subtilis Phage SP-15 Nucleic Acid", Nature New Biology, 239, pp. 70-71 (1972)

^aT. R. Broker et al., "Electron Microscopic Visualization Of tRNA Genes with Ferritin-Avidin: Biotin Labels," Nucl. Acids Res., 5, pp. 363-83 (1978)

^aR. M. K. Dale et al., "Direct Covalent Mercuration of Nucleotides and Polynucleotides," Biochemistry, 14, pp. 2447-57 (1975) ["Dale I"]

^aR. M. K. Dale et al., "The Synthesis and Enzymatic Polymerization of Nucleotides Containing Mercury: Potential Tools for Nucleic Acid Sequencing and Structural Analysis," Proc. Natl. Acad. Sci. USA, 70, pp. 2238-42 (1973) ["Dale II"]

W. S. Dallas and S. Falkow, "Molecular and Genetic Analysis of a DNA Sequence Encoding for Enterotoxin Synthesis in Escherichia Coli," Thirteenth Joint Conference on Cholera, The U.S.-Japan Cooperative Medical Science Program (1979) ["Dallas I"]

W. S. Dallas et al., "The Characterization of an Escherichia Coli Plasmid Determinant That Encodes for the Production of a Heat-Labile Enterotoxin," Plasmids of Medical Environmental and Commercial Importance, K. N. Timmis and A. Puhler, editors, Elsevier/North-Holland Biomedical Press (1979) ["Dallas II"]

^a This document may be relatively more pertinent than other cited documents.

^b This document was cited by the United States patent examiner in the parent application hereto.

D. J. Eckermann and R. H. Symons, "Sequence at the Site of Attachment of an Affinity-Label Derivative of Puromycin on 23-S Ribosomal RNA of Escherichia coli Ribosomes," Eur. J. Biochem., 82, pp. 225-34 (1978)

M. Ya. Feldman, Reactions of Nucleic Acids and Nucleoproteins With Formaldehyde

H. G. Gratzner, "Monoclonal Antibody to 5-Bromo- and 5-Iododeoxyuridine: A New Reagent for Detection of DNA Replication," Science, 218, pp. 474-75 (1982)

M. Grunstein and D. Hogness, "Colony Hybridization: A Method for the Isolation of Cloned DNAs That Contain a Specific Gene", Proc. Natl. Acad. Sci. USA, 72, pp. 3961-65 (1975)

J. L. Guesdon et al., "The Use of Avidin-Biotin Interaction In Immunoenzymatic Techniques", J. Histochem. and Cytochem., 27, pp. 1131-39 (1979)

^aH. Hayashi and K. Nakanishi, "Synthesis and Absolute Configuration of (+)-5-(4',5'-Dihydroxypentyl)uracil from Bacillus Subtilis Phage SP-15 Deoxyribonucleic Acid," J. Am. Chem. Soc., 95, pp. 4081-83 (1973) [Hayashi I]

^aH. Hayashi et al. "Structure and Synthesis of Dihydroxypentyluracil from Bacteriophage SP-15 Deoxyribonucleic Acid," J. Am. Chem. Soc., 95, pp. 8749-57 (1973) [Hayashi II]

K. Hofmann et al., "Iminobiotin Affinity Columns and Their Application To Retrieval of Streptavidin", Proc. Natl. Acad. Sci. USA, 77, pp. 4666-68 (1980)

E. S. Huang and J. S. Pagano, "Nucleic Acid Hybridization Technology and Detection of Proviral Genomes," Methods Virol, 6, pp. 457-97 (1977)

A. M. Jeffrey et al., "Benzo[a] pyrene-Nucleic Acid Derivative Found In Vivo: Structure of a Benzo[a] pyrenetetrahydrodiol Epoxide-Guanosine Adduct," J. Am. Chem. Soc., 98, pp. 5714-15 (1976)

R. G. Kallen et al., "The Occurrence of a New Pyrimidine Base Replacing Thymine in a Bacteriophage DNA: 5-Hydroxymethyl Uracil," J. Mol. Biol., 5, pp. 248-50 (1962)

D. E. Kennell, "Principles and Practices of Nucleic Acid Hybridization", Progr. Nucl. Acid. Res. Mol. Biol., 11, pp. 259-301 (1971)

M. Koreeda et al., "Alkylation of Polyguanylic Acid at the 2-Amino Group and Phosphate by the Potent Mutagen (\pm)-7 β , 8 α -Dihydroxy-9 β ,10 β epoxy -7,8,9,10-tetrahydrobenzo[a]pyrene," J. Am. Chem. Soc., 98, pp. 6720-22 (1976)

^a This document may be relatively more pertinent than other cited documents.

- T. T. Kuo et al., "5-Methylcytosine Replacing Cytosine in the Deoxyribonucleic Acid of a Bacteriophage for Xanthomonas oryzae," J. Mol. Biol., 34, pp. 373-75 (1968)
- a,b P. R. Langer et al., "Enzymatic Synthesis of Biotin-Labeled Polynucleotides: Novel Nucleic Acid Affinity Probes", Proc. Natl. Acad. Sci. USA, 78, pp. 6633-37 (1981) ["Langer I"]
- a,b P. R. Langer and D. C. Ward, "A Rapid and Sensitive Immunological Method For In Situ Gene Mapping," in Developmental Biology Using Purified Genes, ed. D. D. Brown, Academic Press, pp. 647-58 (1981) ["Langer II"]
- a,c P. R. Langer and D. C. Ward, Abstract 1153: "A Rapid and Sensitive Immunological Method For In Situ Gene Mapping," in Journal of Supramolecular Structure and Cellular Biology, ed. Alan R. Liss, Inc. (1981) ["Langer III"]
- I. R. Lehman and E. A. Pratt, "On the Structure of the Glucosylated Hydroxymethylcytosine Nucleotides of Coliphages T2, T4 and T6," J. Biol. Chem. 235, pp. 3254-59 (1960)
- E. T. Maggio (ed.), Enzyme-Immunoassay, pp. 96-07, 169-79 (1980)
- a J. Manning et al., "A Method for Gene Enrichment Based on the Avidin-Biotin Interaction. Application to the Drosophila Ribosomal RNA Genes," Biochemistry, 16, pp. 1364-1370 (1977) ["Manning I"]
- a J. E. Manning et al., "A New Method Of in situ Hybridization," Chromosoma (Berl), 53, pp. 107-117 (1975) ["Manning II"]
- J. Marmur et al., "Unique Properties of Nucleic Acid from Bacillus Subtilis Phage SP-15, Nature New Biology, 239, pp. 68-70 (1972)
- T. W. Munns and M. K. Liszewski, "Antibodies Specific for Modified Nucleotides: An Immunochemical Approach for the Isolation and Characterization of Nucleic Acids," in Progress in Nucleic Acid Research and Molecular Biology, ed. W. E. Cohn, Academic Press, 24, pp. 109-165 (1980)

a This document may be relatively more pertinent than other cited documents.

b This document was cited in the European Patent Office Search Report in an application claiming priority from the parent application, hereto.

c This document was cited by the European patent examiner and by a third party in an application claiming priority from the parent application hereto.

T. Nelson and D. Brutlag, "Addition of Homopolymers to the 3'- Ends of Duplex DNA with Terminal Transferase", Methods in Enzymology-Recombinant DNA, 68, ed. R. Wu, pp. 41-50 (1979)

^aM. Pellegrini et al., "Application of the Avidin-Biotin Method of Gene Enrichment to the Isolation of Long Double-Stranded DNA Containing Specific Gene Sequences", Nucl. Acids Res., 4, pp. 2961-73 (1977)

J. Reiser et al., "Transfer of Small DNA Fragments From Polyacrylamide Gels to Diazobenzoyloxymethyl Paper and Detection by Hybridization With DNA Probes," Biochemical and Biophysical Research Communications, 85, pp. 1104-1112 (1978)

P. W. J. Rigby et al., "Labeling Deoxyribonucleic Acid to High Specific Activity In Vitro by Nick Translation with DNA Polymerase I," J. Mol. Biol., 113, pp. 237-251 (1977)

G. T. Rudkin and B. D. Stollar, "High Resolution Detection of DNA-RNA Hybrids in situ by Indirect Immunofluorescence," Nature, 265, pp. 472-473 (1977)

^{a,b}J. L. Ruth and D. E. Bergstrom, "C-5 Substituted Pyrimidine Nucleosides. 1. Synthesis of C-5 Allyl, Propyl, and Propenyl Uracil and Cytosine Nucleosides via Organo-palladium Intermediates," J. Org. Chem., 43, pp. 2870-2876 (1978)

^aM. So, "Characterization of an *Escherichia coli* Plasmid Encoding for the Synthesis of Heat-Labile Toxin: Molecular Cloning of the Toxin Determinant", Infection and Immunity, 21, pp. 405-411 (1978)

^aA. Sodja and N. Davidson, "Gene Mapping and Gene Enrichment by the Avidin-Biotin Interaction: Use of Cytochrome-C as a Polyamine Bridge," Nucl. Acids Res., 5, pp. 383-399 (1978)

^aM. Swierkowski and D. Shugar, "Poly 5-Ethyluridylic Acid, A Polyuridylic Acid Analogue", J. Mol. Biol., 47, pp. 57-67 (1970)

The Invention

A method of determining the presence or absence of a target in a sample which comprises contacting said sample with at least one compound having the structure:

^a This document may be relatively more pertinent than other cited documents.

^b This document was cited by the United States patent examiner in the parent application hereto.

wherein each of B, B' and B'' represents a purine, deazapurine, or pyrimidine moiety covalently bonded to the C^{1'}-position of the sugar moiety, provided that whenever B, B' or B'' is purine or deazapurine, it is attached at the N⁹-position of the purine or deazapurine, and whenever B, B' or B'' is pyrimidine, it is attached at the N¹-position;

wherein A represents at least one component of a signalling moiety and consists of at least three carbon atoms;

wherein B and A are attached directly or through a linkage group, said linkage group not interfering substantially with the characteristic ability of B to hybridize with said target or of A to produce a detectable signal, wherein if B is purine, A is attached to the 8-position thereof, if B is deazapurine, A is attached to the 7-position thereof, and if B is pyrimidine, A is attached to the 5-position thereof; wherein m, n and p are integers, provided that m and p are

not simultaneously 0 and provided further that n is never 0;
and wherein z represents H- or HO-;

and detecting any signal associated with said compounds
bound to said target.

The Cited Documents

None of the above-cited documents made of record by the applicants herein, either alone or in any combination thereof, renders the claims of this application unpatentable. In the discussion of these documents which follows, each will be referred to, for the sake of simplicity, by its patent number or lead author's name.

Applicants request that these documents: (1) be fully considered by the Examiner during his examination of this application, (2) be listed on the "Notice of References Cited" issued in this application, and (3) be printed on any patent which may issue from this application.

United States patent 3,328,389 refers to a process for preparing nucleotide derivatives, having a purine or a pyrimidine ring as the base and a monophosphorylated-pentose or hexose, containing a protected hydroxy group and a protected phosphoric acid group, as the saccharide moiety. These nucleotides are not modified according to applicants' invention. Compare '389, Formula 2, R_4 , col. 1, lines 40-43, with applicants' dotted line and A groups [claim 125]. The '389 patent's nucleotides do not include a signalling moiety.

United States patent 3,337,530 refers to 3',5' and 2',5'-dinucleoside phosphates in which one of the nucleosides is a 9- β -D-ribofuranosyl-7-deazapurin-5'-yl radical and to a process for their production. None of these nucleosides, inter alia, includes applicants' modified compounds. Compare,

e.g., '530, Formula IVa and IVd, col. 3 and 4, with applicants' compounds [claims 125 and 140].

United States patent 3,338,882 refers to 1- β -D-arabino-furanosylcytosine and 5-substituted-cytosines (a pyrimidine) said to have anti-viral activity. Compare, e.g., '882, Formula I, VII, VIII and XI with applicants' compounds [e.g., claims 125, and 140].

United States patent 3,804,826 refers to thiopyrimidines which are said to possess antiviral activity. Compare '826, Formula 1 (5-substituent = S), with applicants' use of their compounds for the detection of targets [claims 125 and 140].

United States patent 3,893,998 refers to fluorescent analogs of cytosine (a pyrimidine)-containing coenzymes. None is 5-substituted as are the modified cytosine bases of applicants' compounds.

United States patent 3,915,958 refers to 6-substituted purine nucleotides and processes of making them. The compounds are said to modulate the activity of certain enzymes and to possess anti-tumor activity. Compare, e.g., '958, col. 4, line 55 - col. 5, line 11 (Z = halogen), with applicants' claims 125 and 140.

United States patent 3,917,583 refers to 2-substituted derivatives of cyclic AMP (a purine) and processes of making them. The compounds are said to modulate the activity of certain enzymes. Compare, e.g., '583, col. 2, lines 50-63, with claims 125 and 140.

United States patent 3,960,840 refers to fluorescent derivatives of adenine (a purine)-containing compounds and processes of preparing them.

United States patent 3,968,101 refers to 8-substituted purine derivatives. The compounds are said to modulate the

activity of certain enzymes for detecting the presence of targets. Compare, e.g., '101, col. 4, lines 20-30, with applicants' claims 125 and 140.

United States patent 4,008,363 refers to a process for the preparation of nicotinamide 6-(2-hydroxy-3-carboxy-propylamino) purine dinucleotides.

United States patent 4,038,480 refers to the preparation of N⁶-carbamoyl and carbonyl purines that may include an 8-substituent. The compounds are said to modulate the activity of certain enzymes.

United States patent 4,048,307 refers to 8-substituted cyclic adenosine monophosphates (a purine). The compounds are said to be inhibitors of the enzyme phosphodiesterase. Compare, e.g., '307, Formula 1, with applicants' claims 125 and 140.

United States patent 4,086,417 refers to cytidine (a pyrimidine) nucleotide derivatives, inter alia, 5-substituted derivatives. The compound is said to be an antileukemic pharmacological agent. Compare, e.g., '417, formula, col. 2, lines 40-53, with applicants' claims 125 and 140.

United States patent 4,088,639 refers to 6-substituted adenine (a purine) derivatives. Compare, e.g., '639, Col. 1 lines 10-17, with claims 125 and 140.

United States patent 4,096,324 refers to 5'-esters of 1-β-D-arabinofuranosylcytosine (a pyrimidine). These compounds are said to possess anti-viral and antileukemic activity.

United States patent 4,151,349 refers to a potassium salt of β-nicotinamide adenine (a purine) dinucleotide phosphoric acid (β-NADP-K).

United States patents 4,171,432 and 4,213,893 refer to flavin adenine (a purine) dinucleotide-iodothyronine

conjugates. Compare, e.g., '432, col. 2, lines 20-35, with applicants' claims 125 and 140.

United States patent 4,228,237 refers to a method for determining the presence of a ligand in a liquid medium in an immunoassay or receptor binding assay utilizing avidin as a label and a biotin-labeled reagent for the detection of the ligand.

United States patent 4,230,698 refers to 2-substituted arabinofuranosyl nucleosides, including 5-substituted pyrimidines. The compounds are said to have antitumour antimicrobial and antiviral activity. The substituents referred to do not occur on the base of the modified nucleotides, as in the compounds used in applicants' methods.

United States patents 4,134,792 4,230,797, 4,318,980, 4,380,580 and 4,383,031, United States patent application serial number 572,008, German Offenlegungsschriften 2618419 A1 and 2618511 A1, and Great Britain patents 1,548,741 and 1,552,607, refer to a heterogeneous specific binding assay which employs a reactant having activity as a labeling substance in the detection of a ligand in a liquid medium. The method is said to be carried out using a reagent which comprises, as its labeled constituent, a conjugate formed of a specific binding substance coupled to the reactant. The reactant is an enzymatic reactant such as an enzyme substrate or coenzyme. The activity of the conjugated reactant is utilized as means for monitoring the extent of binding of the labeled constituent in conventional heterogeneous specific binding assay schemes. The presence of a ligand in a liquid medium is determined following conventional competitive binding manipulative techniques. The documents refer to use of various derivatives of adenosine (a purine) -5'-phosphates, including 8-[2-(2,4-dinitrophenyl)

aminoethyl] amino adenosine- 5'-triphosphate as reactants. See, e.g., '797 patent, col. 26, lines 49-56. In one example, avidin is the ligand detected and biotin is the specific binding substance. See, e.g., '797, col. 37. These documents do not refer to or suggest the use of any of applicants' compounds for the detection of targets. Compare, e.g., '797, col. 17-col. 30, with applicants' claims 125, and 140.

United States patent 4,247,544 refers to 5-substituted uracil (a pyrimidine) nucleosides. The compounds are said to be antiviral agents. Compare '544, Formula I, Ia and Ib with applicants' claims 125 and 140.

United States patent 4,261,893 refers to bis-phthalimide intermediates. The compounds are said to be useful intermediates for synthesis of chemiluminescent conjugates.

United States patent 4,267,171 refers to 5-substituted cytosine (a pyrimidine) nucleosides. The compounds are said to be antiviral agents. It does not refer to use of applicants' compounds for the detection of targets. Compare, '171, Formula Ia-Ie with applicants' claims 125 and 140.

United States patent 4,318,981 refers to a binding assay employing a labelled conjugate which upon enzymatic cleavage produces a detectable product. Among the labelled conjugates said to be useful in this assay are labelled nucleotides. Compare, e.g., '981, col. 8, lines 30-45, with applicants' claims 125 and 140.

United States patent 4,318,982 refers to a specific binding assay for detecting a ligand in a liquid medium. The assay referred to employs a prosthetic group which when combined with an apoenzyme forms a holoenzyme whose activity is detectable.

United States patent 4,302,204 refers to methods for affixing nucleotides to substrates for subsequent hybridization with labelled DNA probes. This document does not refer to or suggest applicants' method for detecting targets, inter alia, because the presence of targets is detected with radioactively labeled probes, [cols. 4-5].

United States patent 4,358,535 refers to labelled polynucleotide probes (DNA probes) and their use in the diagnosis of pathogens. It refers to methods and compositions involving labeled nucleotide probes complementary to a nucleic acid coding for a characteristic pathogen product. For diagnosis, a clinical sample is said to be treated to liberate the DNA from the microbes present in the sample and the resulting DNA then single-stranded and complexed onto a support. A polynucleotide probe, specific for a DNA sequence characteristic of a suspected pathogen and labeled with a radionuclide, a heavy metal, or a ligand which can serve as a specific binding member to a labelled antibody, fluorescer, chemiluminescer, enzyme, or antibody, is then said to be contacted with the fixed DNA under hybridizing conditions. Detection of hybridization is said to be diagnostic of the presence of the pathogen in the clinical sample. The '535 patent does not refer to or suggest any of applicants' compounds or their use for the detection of targets, inter alia, because the structure of the probes taught differs from those of applicants' methods.

United States patents 4,363,759 and 4,355,165 and United Kingdom patent applications 2,026,690 A, 2,041,922 A and 2,045,239 A refer to chemiluminescent-labeled conjugates and their use in specific binding assays.

United States patent 4,374,925 refers to a method and compositions for performing binding assays involving a

homologous pair said to consist of a ligand and a receptor for the ligand.

United States patent 4,446,231 refers to an immuno-assay wherein the label is said to be an enzyme which converts a precursor into a cycling factor which in turn is interconverted in a cycling detection system. The assay is said to be based on the observation that the enzyme used as the label in the assay may be an enzyme that produces, directly or indirectly, a substance that is capable of influencing a catalytic event, without itself being consumed during the catalytic event.

United States patent 4,469,863 refers to nonionic nucleic acid alkyl and aryl phosphonates and processes for their manufacture and use. The compounds are said to inhibit growth of tumor cells and viruses.

European patent applications 70685 and 70687 refer to DNA hybridization-based methods of diagnosis. The DNA probes said to be used in those methods are labelled by attaching a "light label" at any point on the DNA. See, e.g., 70687, page 6, line 20 - page 7, line 12. The means for effecting such attachment are said to be "documented in the art." Both documents have dates of publication and earliest claimed priority dates (United States applications) subsequent to the effective filing date of the parent application hereto.

German Offenlegungsschrift DE-3045375-A1 refers to 5-substituted pyrimidine nucleosides. It does not suggest applicants' claimed method for use of their compounds for the detection of targets because, inter alia, the R¹ substituents of the '375 application do not include the linkage group signalling moieties, recited by applicants' claim 125.

United Kingdom patent application 2,019,408 A refers to a method for detecting the presence of a DNA fragment in the midst of a complex sample of nucleic acids. The method is said to involve hybridization with an RNA probe modified by an enzyme. The document does not refer to or suggest applicants' claimed use of their compounds for the detection of targets, inter alia, because the enzyme is not attached by the linkage group recited by applicants' claim 125.

United Kingdom patent application 2,034,323 A refers to a method for producing radioactively-labelled DNA probes directed to the genome of viruses causing hepatitis D. This document does not refer to or suggest applicants' methods for using their modified compounds, for the detection of targets, inter alia, because it does not teach the signalling or linkage groups recited by applicants' claim 125.

United Kingdom patent application 2,125,964 A refers to an assay method and probe for polynucleotide sequences. The probes said to be useful in that method are referred to, inter alia, as including both a cDNA sequence substantially complementary to the target sequence and a protein binding sequence adapted to bind to a predetermined protein. The application does not refer to or suggest applicants' claimed method for use of their compounds, for the detection of targets. This document also has a publication date and earliest claimed priority date subsequent to the effective filing date of the parent application hereto.

United Kingdom patent application 2,040 943 A refers to 7-purine nucleotide derivatives, said to be intermediates in the synthesis of favin adenine dinucleotide labeled conjugates for determining the presence of ligands, and where in the 2' and 3' phosphate groups may be cyclized.

Japanese patent application 43-24191 refers (based on the structural formula) to purine nucleotides. It does not refer to applicants' use of any of their compounds for the detection of targets.

Japanese patent application 58-62194 refers (based on its structural formula) to pyrimidines. This document also bears a publication date and a claimed priority date subsequent to the effective filing date of the parent application hereto.

PCT applications W083/02276 and W083/02277 refer to modified ATPs characterized by covalent fixation on the adenine (a purine) ring of a chemical group which may be coupled directly or indirectly with a related molecule, preferably marked by an enzyme, thereby supposedly allowing recognition of the ATP, ADP or AMP. Neither document is a reference because both have publication dates and claimed earliest priority dates subsequent to the effective United States filing date of the parent application hereto.

PCT application W083/02286 refers to a method for detecting the presence of a nucleic acid sequence by contacting a composition with a probe containing a complementary nucleic acid sequence and carrying at least a N-2-acetylaminofluorene group fixed covalently to at least one base. The presence of the nucleic acid sequence is said to be revealed by antibodies to N-2-(guanosine-8-yl)-acetyl amino fluorene. The '286 application does not refer to or suggest applicants' use of their compounds for the detection of targets, because, inter alia, the acetylaminofluorene group is attached to the N² position of the bases of the probe, rather than to the positions specified in applicants' claim 125. This document is not a reference

because it has a publication date subsequent to the effective U.S. filing date of the parent application hereto.

Bauman I and Bauman II refer to fluorochrome-labeled RNA which is said to detect in situ hybrids without the long exposure times required in the autoradiographical hybridization methods. Bauman I and II's label is attached to the residue of the sugar moiety rather than to the nucleotide bases as in applicants' compounds.

Bayer refers to the high affinity constant between avidin and biotin. Bayer also states that the avidin-biotin complex represents a complementary approach to, and/or a potential replacement for, lectins and antibodies in biological interactions that exploit the specific binding between a protein and a ligand.

Bergstrom I and Bergstrom II refer to 5-substituted pyrimidine nucleosides and methods of making them. Compare, e.g., Bergstrom I, Scheme 1 (page 8108), with applicants' claims 125 and 140.

Bigge refers to a method for alkylation of uracil (a pyrimidine) nucleosides and monophosphate nucleotides to produce olefinic and saturated substituents substituted at the 5-position. Bigge does not refer to or suggest applicants' claimed use of their compounds for the detection of targets. Compare, Bigge, Scheme 1 (page 2033), with applicants' claims 125 and 140.

Brandon, Hayashi I, Hayashi II and Marmur refer to the synthesis of (+)-5-(4',5'-dihydroxypentyl)uracil (a pyrimidine), a base which is said to replace thymine in bacteriophage SP-15 DNA. This base, or natural DNA in which it occurs, does not refer to or suggest applicants' claimed compounds. Compare, e.g., Hayashi II, Formula I (page 8750), with applicants'

claims 125 and 140. These documents also do not refer to or suggest applicants' use of their compounds for the detection of targets because, inter alia, these documents only refer to bases without sugars. See, e.g., Hayashi II, page 8750, and because the bases referred to do not include signaling moieties attached by linkage groups.

Broker refers to a method for indirect electron microscopic visualization and mapping of tRNA and other short transcripts hybridized to DNA. This method is said to depend upon the attachment of the electron-dense protein ferritin to the RNA, binding being mediated by the avidin-biotin complex. Broker first refers to covalently attaching biotin to the 3' end of tRNA using an $\text{NH}_2(\text{CH}_2)_5\text{NH}_2$ bridge. The resulting tRNA-biotin adduct is then said to be hybridized to complementary DNA sequences present in a single stranded non-homology loop of a DNA:DNA heteroduplex. Avidin, covalently crosslinked to ferritin, is then mixed with the heteroduplex and becomes bound to the hybridized tRNA-biotin for electron microscopic visualization of the hybrid. Since in the method of Broker, the biotin is said to be attached to adenine (a purine) at the N⁹ position, it does not refer to or suggest applicants' use of their compounds for the detection of targets. Compare, Broker, Figure 1, with applicants' claims 125 and 140.

Dale I and Dale II refer to methods of synthesizing nucleotides containing mercury atoms. The mercury atoms are said to be attached to the 5-position of pyrimidine nucleotides or to the 7-position of 7-deazapurine nucleotides. See, e.g., Dale I, Figure 1 (page 2450); Dale II, Figure 2 (page 2239). Such nucleotides in the presence of mercaptans are also said

to be substrates for incorporation into oligo- or polynucleotides. Compare, Dale I, Figure 1 (page 2239) and Dale II, Figure 2 (page 2450), with applicants' claims 125 and 140.

Dallas I, Dallas II, and So refer to a filter blot hybridization-based method for detecting the presence of a specific DNA in a clinical sample. The DNA probe said to be employed in this method is a ^{32}P -labelled, cloned, heat-labile toxin (LT) of enteropathogenic E.coli. Dallas I and II and So do not refer to or suggest applicants' use of their compounds for the detection of targets.

Eckermann refers to an affinity-label on 23S ribosomal RNA. As depicted in Figure 1, the Eckermann label is on the sugar moiety, not the base, as recited by of applicants' claims.

Feldman refers to the chemical modification of nucleic acids, particularly modifications with formaldehyde. These modifications are said to occur at the NH or NH_2 positions of the bases (e.g., pages 3 and 7) and do not involve applicants' linkage groups.

Gratzner refers to the production and use of monoclonal antibodies said to be specific for 5-bromodeoxyuridine. It does not refer to or suggest applicants' use of their compounds for the detection of targets. This document is not a reference because its publication date is subsequent to the effective United States filing date of the parent application hereto.

Grunstein refers to the screening of hybrid plasmid-containing colonies with specific DNA probes. The probes referred to by Grunstein are radiolabelled. Grunstein does not refer to or suggest applicants' use of their compounds for the detection of targets.

Guesdon and Hofmann refer to the use of the avidin-biotin complex in assay techniques employing labeled antibodies and peptides.

Huang refers to methods for detecting pathogens using RNA-DNA and DNA-DNA hybridizations. The DNA or RNA probes said to be used in such detection methods are ^3H or ^{125}I -labeled.

Jeffrey refers to benzo[a]pyrene -nucleic acid derivatives. These derivatives do not suggest or refer to applicants' use of their compounds for the detection of targets. Compare, e.g., Jeffrey, Formula (pages 5714), with applicants' claim 125.

Kallen refers to 5-hydroxymethyl uracil, a pyrimidine base from Bacillus subtilis bacteriophage SP8.

Kennell refers to the practice of nucleic acid hybridization. It is cited for background.

Koreeda refers to the alkylation of polyguanylic acid at the 2-amino group and phosphate. It does not refer to or suggest any of applicants' claimed subject matter. Compare, e.g., Koreeda, Figure 4 (page 6721), with applicants' claim 125.

Kuo refers to 5-methyl cytosine, a pyrimidine base in the DNA of a bacteriophage for Xanthomonas oryzae.

Langer I refers to modified compounds and nucleotides in accordance with this invention. The publication date of this document (November 1981) is subsequent to the effective filing date of the parent application hereto.

Langer II and III refer to modified compounds and nucleotides in accordance with this invention. Langer II, even if "published" prior to the effective filing date of this application, and Langer III, or other like seminars,

even if orally presented prior to the effective filing date of this application, are not prior art to it because, inter alia, they are not "of another" and were not printed publications more than one year before the effective filing date of this application.

Moreover, as demonstrated by the Declaration Under 37 C.F.R. § 1.131 of David C. Ward, Penina R. Langer - Safer And Alexander Waldrop III submitted together with the Amendment Under 37 C.F.R. § 1.116 And Statement Under 37 C.F.R. § 1.56 and 1.99 on June 16, 1987 in the parent application hereto, the subject matter referred to in Larger III was completed by applicants prior to the publication date of Larger III, as well as the date of an oral presentation of substantially the same subject matter at Rockefeller University on December 11, 1980.

Lehman refers to the structure of glucosylated hydroxymethyl cytosine (a pyrimidine) nucleotides of Coliphages T2, T4 and T6. These naturally occurring DNAs do not refer to or suggest applicants' use of their compounds for the detection of targets because, inter alia, they do not include applicants' specific labels.

Maggio refer to general methods of enzyme-protein coupling and the detection of the resulting conjugates. It is cited for background.

Manning I, Manning II and Pellegrini refer to methods of preparing a modified RNA having a biotin attached via a cytochrome C bridge. These documents refer to hybridizing this modified RNA to DNA and to detecting the resulting RNA-DNA hybrid by binding the biotin to avidin on a solid support. Manning I and II's and Pellegrini's methods of attaching the cytochrome C/biotin conjugate to the RNA do not result in

attachment at the 7-deazapurine or 5-pyrimidine positions, as required by applicants' claims. Instead, it results in attachment to an NH or NH₂ on the base because formaldehyde is used. See, e.g., Manning II, p. 108, and Feldman, supra.

Munns refers to antibodies specific for modified nucleosides. Munns' antibodies are directed to bases, rather than to a signalling moiety attached, by way of a linkage group, to a base. See claims 125 and 100. Compare. e.g., Munns, Table I (page 112), with applicants' claim 155.

Reiser refers to methods for detecting specific DNA fragments on nitrocellulose filters using ³²P-labelled DNA probes.

Rigby refers to methods for labelling DNA to high specific activity employing DNA polymerase I and ³²P-labelled triphosphates. Nelson refers to methods of adding homopolymers to the 3' ends of DNA with terminal transferase.

Rudkin refers to a method for detecting RNA-DNA hybrids by indirect immunofluorescence using antibodies against nucleic acid.

Ruth refers to methods of producing 5-allyluridine and cytidine and 5-allyl-2'-deoxyuridine and deoxycytidine, (both pyrimidines) and the hydrogenation of those compounds to 5-propyl uridines, deoxyuridines and cytidines and deoxycytidines, respectively. C-5 substituted pyrimidines are said to possess anti-viral and enzyme inhibitory properties. Compare, Ruth's 5-allyl and 5-propyl substituents with those of claim 125 and 140. Also, Ruth's compounds do not include the signalling moiety of applicants' claim 125.


Sodja refers to methods of coupling biotin through a cytochrome C bridge to the oxidized 2', 3' terminus of an RNA sugar ring. It then refers to the use of such modified

bases in detecting RNA/DNA hybrids. None of Sodja's compounds suggests applicants' claims, inter alia, because none of Sodja's compounds have a substituent in the positions recited by applicants' claims 125 and 140.

Swierkowski refers to poly 5-ethyluridylic (a pyrimidine) acid and a method for preparing it. Compare, e.g., Swierkowski's 5-ethyl substituent with applicants' claims 125 and 140.

Applicants request early allowance of the amended claims pending in this application.

Respectfully submitted,


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